

Figure 1. Nucleotide and amino acid sequence of 60kCRMP from *Chlamydia muridum*.

atg cga ata gga gat cct atg aac aaa ctc atc aga cga gct gtg acg	48
Met Arg Ile Gly Asp Pro Met Asn Lys Leu Ile Arg Arg Ala Val Thr	
1 5 10 15	
atc ttc gcg gtg act agt gtg gcg agt tta ttt gct agc ggg gtg tta	96
Ile Phe Ala Val Thr Ser Val Ala Ser Leu Phe Ala Ser Gly Val Leu	
20 25 30	
gag acc tct atg gca gag tct ctc tct acc aac gtt att agc tta gct	144
Glu Thr Ser Met Ala Glu Ser Leu Ser Thr Asn Val Ile Ser Leu Ala	
35 40 45	
gac acc aaa gcg aaa gag acc act tct cat caa aaa gac aga aaa gca	192
Asp Thr Lys Ala Lys Glu Thr Thr Ser His Gln Lys Asp Arg Lys Ala	
50 55 60	
aga aaa aat cat caa aat agg act tcc gta gtc cgt aaa gag gtt act	240
Arg Lys Asn His Gln Asn Arg Thr Ser Val Val Arg Lys Glu Val Thr	
65 70 75 80	
gca gtt cgt gat act aaa gct gta gag cct aga cag gat tct tgc ttt	288
Ala Val Arg Asp Thr Lys Ala Val Glu Pro Arg Gln Asp Ser Cys Phe	
85 90 95	
ggc aaa atg tat aca gtc aaa gtt aat gat gat cgt aat gta gaa atc	336
Gly Lys Met Tyr Thr Val Lys Val Asn Asp Asp Arg Asn Val Glu Ile	
100 105 110	
gtg cag tcc gtt cct gaa tat gct acg gta gga tct cca tat cct att	384
Val Gln Ser Val Pro Glu Tyr Ala Thr Val Gly Ser Pro Tyr Pro Ile	
115 120 125	
gag att act gct ata ggg aaa aga gac tgt gtt gat gta atc att aca	432
Glu Ile Thr Ala Ile Gly Lys Arg Asp Cys Val Asp Val Ile Ile Thr	
130 135 140	
cag caa tta cca tgc gaa gca gag ttt gtt agc agt gat cca gct act	480
Gln Gln Leu Pro Cys Glu Ala Glu Phe Val Ser Ser Asp Pro Ala Thr	
145 150 155 160	
act cct act gct gat ggt aag cta gtt tgg aaa att gat cgg tta gga	528
Thr Pro Thr Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg Leu Gly	
165 170 175	
cag ggc gaa aag agt aaa att act gta tgg gta aaa cct ctt aaa gaa	576
Gln Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu	
180 185 190	
ggg tgc tgc ttt aca gct gca acg gtt tgt gct tgt cca gag atc cgt	624
Gly Cys Cys Phe Thr Ala Ala Thr Val Cys Ala Cys Pro Glu Ile Arg	
195 200 205	
tcg gtt acg aaa tgt ggc cag cct gct atc tgt gtt aaa cag gaa ggt	672
Ser Val Thr Lys Cys Gly Gln Pro Ala Ile Cys Val Lys Gln Glu Gly	
210 215 220	
cca gaa agc gca tgt ttg cgt tgc cca gta act tat aga att aat gta	720
Pro Glu Ser Ala Cys Leu Arg Cys Pro Val Thr Tyr Arg Ile Asn Val	
225 230 235 240	

gtc aac caa gga aca gca aca gca cgt aat gtt gtt gtg gaa aat cct Val Asn Gln Gly Thr Ala Thr Ala Arg Asn Val Val Val Glu Asn Pro 245 250 255	768
gtt cca gat ggc tat gct cat gca tcc gga cag cgt gta ttg aca tat Val Pro Asp Gly Tyr Ala His Ala Ser Gly Gln Arg Val Leu Thr Tyr 260 265 270	816
act ctt ggg gat atg caa cct gga gaa cag aga aca atc acc gtg gag Thr Leu Gly Asp Met Gln Pro Gly Glu Gln Arg Thr Ile Thr Val Glu 275 280 285	864
ttt tgt ccg ctt aaa cgt ggt cga gtc aca aat att gct aca gtt tct Phe Cys Pro Leu Lys Arg Gly Arg Val Thr Asn Ile Ala Thr Val Ser 290 295 300	912
tac tgt ggt gga cac aaa aat act gct agc gta aca aca gtg atc aat Tyr Cys Gly Gly His Lys Asn Thr Ala Ser Val Thr Thr Val Ile Asn 305 310 315 320	960
gag cct tgc gtg caa gtt aac atc gag gga gca gat tgg tct tat gtt Glu Pro Cys Val Gln Val Asn Ile Glu Gly Ala Asp Trp Ser Tyr Val 325 330 335	1008
tgt aag cct gta gaa tat gtt atc tct gtt tct aac cct ggt gac tta Cys Lys Pro Val Glu Tyr Val Ile Ser Val Ser Asn Pro Gly Asp Leu 340 345 350	1056
gtt tta cga gac gtt gta att gaa gat acg ctt tct cct gga ata act Val Leu Arg Asp Val Val Ile Glu Asp Thr Leu Ser Pro Gly Ile Thr 355 360 365	1104
gtt gtt gaa gca gct gga gct cag att tct tgt aat aaa ttg gtt tgg Val Val Glu Ala Ala Gly Ala Gln Ile Ser Cys Asn Lys Leu Val Trp 370 375 380	1152
act ttg aag gaa ctc aat cct gga gag tct tta caa tat aag gtt cta Thr Leu Lys Glu Leu Asn Pro Gly Glu Ser Leu Gln Tyr Lys Val Leu 385 390 395 400	1200
gta aga gct caa act cca ggg caa ttc aca aac aac gtt gtt gtg aaa Val Arg Ala Gln Thr Pro Gly Gln Phe Thr Asn Asn Val Val Val Lys 405 410 415	1248
agt tgc tct gat tgc ggt att tgt act tct tgc gca gaa gca aca act Ser Cys Ser Asp Cys Gly Ile Cys Thr Ser Cys Ala Glu Ala Thr Thr 420 425 430	1296
tac tgg aaa gga gtt gct gct act cat atc ttc gta gta gat act tgt Tyr Trp Lys Gly Val Ala Ala Thr His Met Cys Val Val Asp Thr Cys 435 440 445	1344
gat cct att tgc gta gga gag aac act gtt tat cgt atc tgt gtg aca Asp Pro Ile Cys Val Gly Glu Asn Thr Val Tyr Arg Ile Cys Val Thr 450 455 460	1392
aac aga ggt tct gct gaa gat aca aat gtg tcc tta att ttg aaa ttc Asn Arg Gly Ser Ala Glu Asp Thr Asn Val Ser Leu Ile Leu Lys Phe 465 470 475 480	1440
tct aaa gaa tta caa cct ata tct ttc tct gga cca act aaa gga acc Ser Lys Glu Leu Gln Pro Ile Ser Phe Ser Gly Pro Thr Lys Gly Thr 485 490 495	1488

485										490					495					
att	aca	gga	aac	acg	gta	gtg	ttt	gat	tcg	tta	cct	aga	tta	ggt	tct	1536				
Ile	Thr	Gly	Asn	Thr	Val	Val	Phe	Asp	Ser	Leu	Pro	Arg	Leu	Gly	Ser					
			500					505						510						
aaa	gaa	act	gta	gag	ttt	tct	gta	acg	ttg	aaa	gca	gta	tcc	gct	gga	1584				
Lys	Glu	Thr	Val	Glu	Phe	Ser	Val	Thr	Leu	Lys	Ala	Val	Ser	Ala	Gly					
		515					520					525								
gat	gct	cgt	ggg	gaa	gct	att	ctt	tct	tcc	gat	aca	ttg	aca	gtt	cct	1632				
Asp	Ala	Arg	Gly	Glu	Ala	Ile	Leu	Ser	Ser	Asp	Thr	Leu	Thr	Val	Pro					
		530				535					540									
gta	tct	gat	acg	gag	aat	aca	cat	atc	tat							1662				
Val	Ser	Asp	Thr	Glu	Asn	Thr	His	Ile	Tyr											
545						550														

Figure 2 *C. trachomatis* equivalent 60kCRMP nucleic acid and amino acid sequence.

atg cga ata gga gat cct atg aac aaa ctc atc aga cga gca gtg acg	48
Met Arg Ile Gly Asp Pro Met Asn Lys Leu Ile Arg Arg Ala Val Thr	
1 5 10 15	
atc ttc gcg gtg act agt gtg gcg agt tta ttt gct agc ggg gtg tta	96
Ile Phe Ala Val Thr Ser Val Ala Ser Leu Phe Ala Ser Gly Val Leu	
20 25 30	
gag acc tct atg gca gag tct ctc tct aca aac gtt att agc tta gct	144
Glu Thr Ser Met Ala Glu Ser Leu Ser Thr Asn Val Ile Ser Leu Ala	
35 40 45	
gac acc aaa gcg aaa gac aac act tct cat aaa agc aaa aaa gca aga	192
Asp Thr Lys Ala Lys Asp Asn Thr Ser His Lys Ser Lys Lys Ala Arg	
50 55 60	
aaa aac cac agc aaa gag act ccc gta gac cgt aaa gag gtt gct ccg	240
Lys Asn His Ser Lys Glu Thr Pro Val Asp Arg Lys Glu Val Ala Pro	
65 70 75 80	
gtt cat gag tct aaa gct aca gga cct aaa cag gat tct tgc ttt ggc	288
Val His Glu Ser Lys Ala Thr Gly Pro Lys Gln Asp Ser Cys Phe Gly	
85 90 95	
aga atg tat aca gtc aaa gtt aat gat gat cgc aat gtt gaa atc aca	336
Arg Met Tyr Thr Val Lys Val Asn Asp Asp Arg Asn Val Glu Ile Thr	
100 105 110	
caa gct gtt cct gaa tat gct acg gta gga tct ccc tat cct att gaa	384
Gln Ala Val Pro Glu Tyr Ala Thr Val Gly Ser Pro Tyr Pro Ile Glu	
115 120 125	
att act gct aca ggt aaa agg gat tgt gtt gat gtt atc att act cag	432
Ile Thr Ala Thr Gly Lys Arg Asp Cys Val Asp Val Ile Ile Thr Gln	
130 135 140	
caa tta cca tgt gaa gca gag ttc gta cgc agt gat cca gcg aca act	480
Gln Leu Pro Cys Glu Ala Glu Phe Val Arg Ser Asp Pro Ala Thr Thr	
145 150 155 160	
cct act gct gat ggt aag cta gtt tgg aaa att gac cgc tta gga caa	528
Pro Thr Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg Leu Gly Gln	
165 170 175	
ggc gaa aag agt aaa att act gta tgg gta aaa cct ctt aaa gaa ggt	576
Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly	
180 185 190	
tgc tgc ttt aca gct gca aca gta tgc gct tgt cca gag atc cgt tgc	624
Cys Cys Phe Thr Ala Ala Thr Val Cys Ala Cys Pro Glu Ile Arg Ser	
195 200 205	
gtt aca aaa tgt gga caa cct gct atc tgt gtt aaa caa gaa ggc cca	672
Val Thr Lys Cys Gly Gln Pro Ala Ile Cys Val Lys Gln Glu Gly Pro	
210 215 220	
gag aat gct tgt ttg cgt tgc cca gta gtt tac aaa att aat ata gtg	720
Glu Asn Ala Cys Leu Arg Cys Pro Val Val Tyr Lys Ile Asn Ile Val	
225 230 235 240	

aac caa gga aca gca aca gct cgt aac gtt gtt gtt gaa aat cct gtt Asn Gln Gly Thr Ala Thr Ala Arg Asn Val Val Val Glu Asn Pro Val 245 250 255	768
cca gat ggt tac gct cat tct tct gga cag cgt gta ctg acg ttt act Pro Asp Gly Tyr Ala His Ser Ser Gly Gln Arg Val Leu Thr Phe Thr 260 265 270	816
ctt gga gat atg caa cct gga gag cac aga aca att act gta gag ttt Leu Gly Asp Met Gln Pro Gly Glu His Arg Thr Ile Thr Val Glu Phe 275 280 285	864
tgt ccg ctt aaa cgt ggt cgt gct acc aat ata gca acg gtt tct tac Cys Pro Leu Lys Arg Gly Arg Ala Thr Asn Ile Ala Thr Val Ser Tyr 290 295 300	912
tgt gga gga cat aaa aat aca gca agc gta aca act gtg atc aac gag Cys Gly Gly His Lys Asn Thr Ala Ser Val Thr Val Ile Asn Glu 305 310 315 320	960
cct tgc gta caa gta agt att gca gga gca gat tgg tct tat gtt tgt Pro Cys Val Gln Val Ser Ile Ala Gly Ala Asp Trp Ser Tyr Val Cys 325 330 335	1008
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ttg cga gat gtc gtc gtt gaa gac act ctt tct ccc gga gtc aca gtt Leu Arg Asp Val Val Val Glu Asp Thr Leu Ser Pro Gly Val Thr Val 355 360 365	1104
ctt gaa gct gca gga gct caa att tct tgt aat aaa gta gtt tgg act Leu Glu Ala Ala Gly Ala Gln Ile Ser Cys Asn Lys Val Val Trp Thr 370 375 380	1152
gtg aaa gaa ctg aat cct gga gag tct cta cag tat aaa gtt cta gta Val Lys Glu Leu Asn Pro Gly Glu Ser Leu Gln Tyr Lys Val Leu Val 385 390 395 400	1200
aga gca caa act cct gga caa ttc aca aat aat gtt gtt gtg aag agc Arg Ala Gln Thr Pro Gly Gln Phe Thr Asn Asn Val Val Val Lys Ser 405 410 415	1248
tgc tct gac tgt ggt act tgt act tct tgc gca gaa gcg aca act tac Cys Ser Asp Cys Gly Thr Cys Thr Ser Cys Ala Glu Ala Thr Thr Tyr 420 425 430	1296
tgg aaa gga gtt gct gct act cat atg tgc gta gta gat act tgt gac Trp Lys Gly Val Ala Ala Thr His Met Cys Val Val Asp Thr Cys Asp 435 440 445	1344
cct gtt tgt gta gga gaa aat act gtt tac cgt att tgt gtc acc aac Pro Val Cys Val Gly Glu Asn Thr Val Tyr Arg Ile Cys Val Thr Asn 450 455 460	1392
aga ggt tct gca gaa gat aca aat gtt tct tta atg ctt aaa ttc tct Arg Gly Ser Ala Glu Asp Thr Asn Val Ser Leu Met Leu Lys Phe Ser 465 470 475 480	1440
aaa gaa ctg caa cct gta tcc ttc tct gga cca act aaa gga acg att Lys Glu Leu Gln Pro Val Ser Phe Ser Gly Pro Thr Lys Gly Thr Ile	1488

485	490	495	
aca ggc aat aca gta gta ttc gat tcg tta cct aga tta ggt tct aaa			1536
Thr Gly Asn Thr Val Val Phe Asp Ser Leu Pro Arg Leu Gly Ser Lys			
500	505	510	
gaa act gta gag ttt tct gta aca ttg aaa gca gta tca gct gga gat			1584
Glu Thr Val Glu Phe Ser Val Thr Leu Lys Ala Val Ser Ala Gly Asp			
515	520	525	
gct cgt ggg gaa gcg att ctt tct tcc gat aca ttg act gtt cca gtt			1632
Ala Arg Gly Glu Ala Ile Leu Ser Ser Asp Thr Leu Thr Val Pro Val			
530	535	540	
tct gat aca gag aat aca cac atc tat			1659
Ser Asp Thr Glu Asn Thr His Ile Tyr			
545	550		

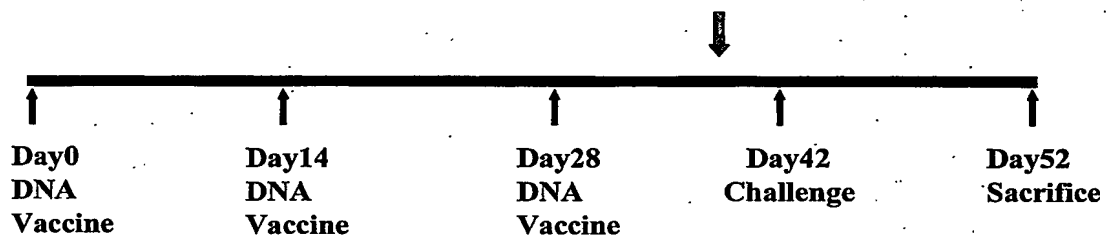
Figure 3 Immunization protocol.

Protocol

Animal: Female BALB/c mice(4-5weeks old)

: Four to 8 mice per group

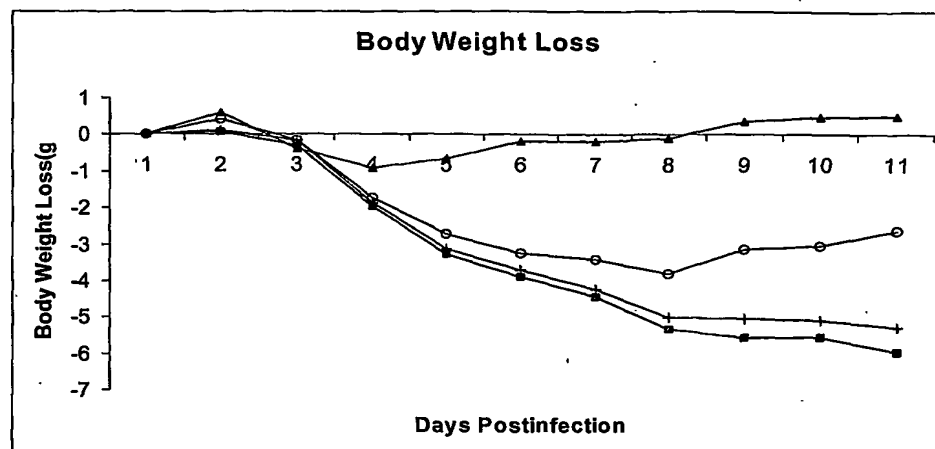
**Before challenge
collect serum**



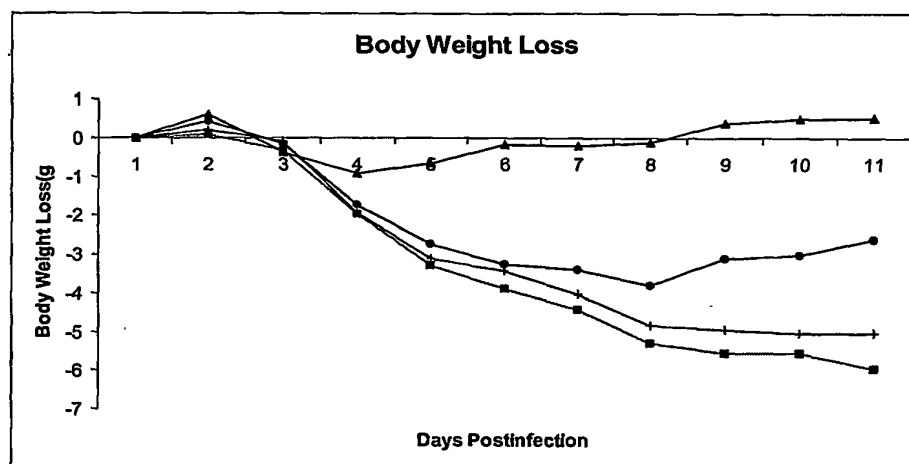
DNA Vaccine: Intranasal 100ug&intramuscular 200ug of plasmid DNA(2ug/ul)

Figure 4 Body Weight loss after immunization.

Panel A 60kCRMP full-length



Panel B 60kCRMP signal sequence deleted



▲ - EB

○ - pCACT CRMP60k

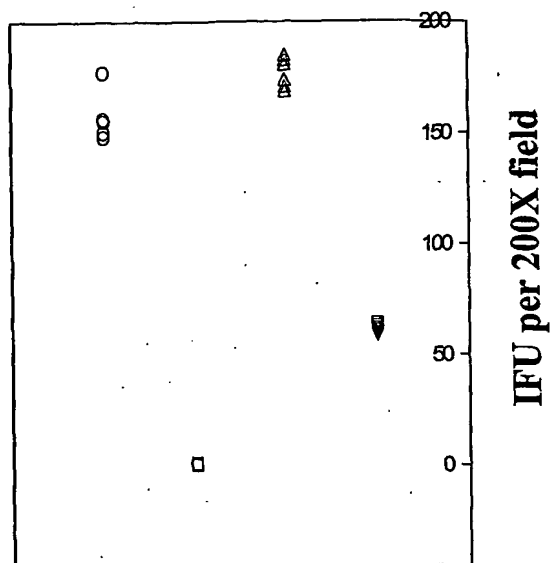
● - pCACT CRMPdelta

+ - pCAMycHis

■ - Naive

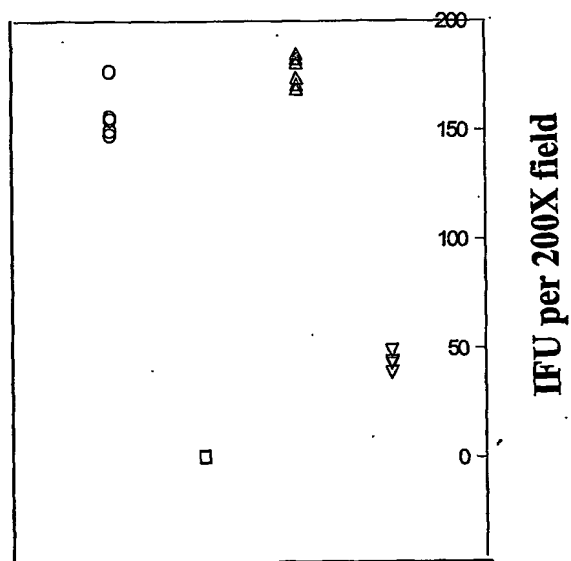
Figure 5 Clearance of Chlamydia from the Lungs of Immunized Mice.

Panel A 60kCRMP full-length



(p<0.001)

Panel B 60kCRMP signal sequence deleted



(p<0.001)

○ - Naïve,

□ - EB,

Δ - pCAMycHis,

▽ - pCACT CRMP60k (Panel A)

- pCACT CRMPdelta

Figure 6. Plasmid pET30b(+)-60kCRMP+SP cloning Procedure.

